1645

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12/4/02

Attorney's Docket No. B00192.70034.US

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Elma Tchilian and Peter Beverley
Serial No.	:	10/020,758
Filed	:	October 30, 2001
Conf. No.:	:	8645
For	:	SCREENS FOR SUSCEPTIBILITY TO IMMUNODEFICIENCY AND VIRAL DISEASE
Examiner	:	Unknown
Art Unit	:	1645

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

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Heather B. Hill

Commissioner for Patents
Washington, D.C. 20231

Sir:

Transmitted herewith are the following documents:

[X] Letter to Commissioner
[X] Certified copy of priority document UK apl. no. 0114512.7, filed June 14, 2001
[X] Return Receipt Postcard

If the enclosed papers are considered incomplete, the Mail Room and/or the Application Branch is respectfully requested to contact the undersigned at (617) 720-3500, Boston, Massachusetts.

No check is enclosed. If a fee is determined to be required, the balance may be charged to the account of the undersigned, Deposit Account No. 23/2825. A duplicate of this sheet is enclosed.

Respectfully submitted,

By: John R. Van Amsterdam

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Docket No. B0192.70034.US
Dated: November 13, 2002
xNDD

658386.1

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Heather B. Hill

Commissioner for Patents
Washington, D.C. 20231

LETTER TO COMMISSIONER

Sir:

Enclosed is a certified copy of the foreign priority document listed below:

UK apl. no. 0114512.7, filed June 14, 2001 .

If any other information is needed, please contact the undersigned attorney by phone to expedite the further prosecution of this patent application.

Respectfully submitted,

By: _____

John R. Van Amsterdam, Reg No. 40,212
Wolf, Greenfield & Sacks, P.C.
600 Atlantic Avenue
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Docket No. B0192.70034.US
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Signed R. Mahoney

Dated 22 October 2002

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14 JUN 2001

15JUN01 E637331-6 D02882
P01/7700 0.00-0114512.7

1.	Your reference	SCB/NLW/57751/000
2.	Patent application number *(The Patent Office will fill in this part)*	0114512.7
3.	Full name, address and postcode of the or of each applicant *(underline all surnames)*	The Edward Jenner Institute for Vaccine Research Compton Newbury Berkshire RG20 7NN
	Patents ADP number *(if you know it)*	8166461300 1
	If the applicant is a corporate body, give the country/state of its incorporation	United Kingdom
4.	Title of the invention	Screens for Susceptibility To Immunodeficiency and Viral Disease
5.	Name of your agent *(if you have one)*	BOULT WADE TENNANT
	"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*	VERULAM GARDENS 70 GRAY'S INN ROAD LONDON WC1X 8BT
	Patents ADP number *(if you know it)*	42001

6.	If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number	Country	Priority application number (if you know it)	Date of filing *(day/month/year)*

7.	If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application	Date of filing *(day / month / year)*

8.	Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:* *a) any applicant named in part 3 is not an inventor, or* *b) there is an inventor who is not named as an applicant, or* *c) any named applicant is a corporate body.* *See note (d))*	YES

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Drawing(s)	1 + 1

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Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	
Request for preliminary examination and search *(Patents Form 9/77)*	
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(Please specify)*	

11 I/We request the grant of a patent on the basis of this application.

Signature: Boult Wade Tennant

Date: 14 June 2001

12. Name and daytime telephone number of person to contact in the United Kingdom: **Claire Baldock 020 7430 7500**

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SCREENS FOR SUSCEPTIBILITY TO IMMUNODEFICIENCY AND VIRAL DISEASE

Field of the invention

The invention relates to associations between genetic variation in the gene encoding CD45 and human disease. In particular the invention provides methods of screening human subjects for susceptibility to viral disease and/or a predisposition to developing more severe viral disease and also methods of screening human subjects for susceptibility to developing immunodeficiency and/or a predisposition to developing more severe immunodeficiency.

Background

The leucocyte common antigen CD45 is an abundant tyrosine phosphatase, expressed on all leucocytes (Trowbridge, I. S., and M. L. Thomas. 1994. Ann. Rev. Immunol. 12:85). The phosphatase activity of CD45 is essential for lymphocyte antigen receptor signal transduction. Both CD45 deficient mice (Kishihara, K. *et al.*, 1993. Cell 74:143; Byth, K. et al. 1996. J. Exp. Med. 183:170) and humans (Kung, C. *et al.*, 2000, Nature Medicine, 6: 343; Tchilian, E. Z. *et al.*, 2001, J. Immunol., 166: 1308) are severely immunodeficient, with very few peripheral T lymphocytes and impaired T and B cell responses.

Multiple CD45 isoforms can be generated by alternative splicing of exons A, B, and C of the extracellular domain (Saga, Y. *et al.*, 1986. Proc Natl Acad Sci USA, 83: 6940; Streuli, M. *et al.*, 1987, J. Exp. Med., 166: 1548). In humans, naive T cells express high molecular weight CD45 isoforms, recognised by CD45RA monoclonal antibodies (mAbs), but activation of the cells results in a change to

expression of low molecular weight isoforms, detected by a CD45RO mAb (Akbar, A. N., *et al.*, 1988, J. Immunol., 140: 2171). These two major subsets of T lymphocytes, expressing CD45RA and CD45RO have been termed naive and memory cells.

Genetically determined abnormal CD45 splicing has been described in humans (Schwinzer, R., and K. Wonigeit, 1990, J. Exp. Med. 171:1803.). Activated or memory lymphocytes in these individuals continue to express both high and low molecular weight CD45 isoforms in contrast to the normal pattern of low molecular weight isoform expression. A C to G transversion at position 77 (C77G) in the fourth or A exon of the gene encoding CD45, has been shown to prevent the normal splicing of this exon in the affected individuals (Thude, H. *et al.*, 1995, Eur. J. Immunol., 25: 2101; Zilch, C. F. *et al.*, 1998, Eur. J. Immunol., 28: 22) by disrupting a strong exonic splicing silencer (Lynch, K. W. a. W., 2001, J. Biol. Chem). The C77G polymorphism has been shown to correlate with development of multiple sclerosis in some families (Jacobsen, M. *et al.*, 2000, Nat Genet, 26: 495) suggesting that the regulation of CD45 alternative splicing may be important in the development of some autoimmune disorders.

The present inventors have investigated the pattern of CD45 expression in HIV infection and have demonstrated a statistically significant association between the C77G mutation and HIV-1 infection.

Further observations made by the present inventors provide evidence that the C77G mutation may be a marker for general susceptibility to viral infection and/or a marker for disease severity

following viral infection. Accordingly, the inventors have developed screens for determining susceptibility of human subjects to viral infection and/or identifying individuals pre-disposed to developing more severe disease following viral infection based on screening for the presence or absence of the C77G mutation at the protein, mRNA or genomic DNA level.

Therefore, in accordance with a first aspect of the invention there is provided a method of screening a human subject for susceptibility to viral infection and/or pre-disposition to developing severe disease following viral infection, which method comprises screening for the presence or absence in the genome of the subject of one or more polymorphic variants or mutations in the gene encoding CD45 or of one or more polymorphic variants in linkage disequilibrium with or in close physical proximity to a polymorphic locus in the gene encoding CD45.

In the context of this application the terms "gene encoding CD45" and "CD45 gene" are used interchangeably and refer to a gene, also referred to as the PTPRC gene located at gene map locus 1q31-32 (OMIM accession 151460). The complete sequence of the gene is available via publicly accessible genome sequence databases. A list of GenBank accession numbers for individual exons of the gene is provided.

The method of the invention preferably comprises screening the genome of the individual for one or more polymorphic variants of the gene encoding CD45 which have previously been demonstrated to show statistically significant association with susceptibility to viral disease and/or severity of viral disease, for example in a population-based genetic association study.

The invention also contemplates screens based on polymorphic variants (whether or not within the CD45 gene) which have not themselves been shown to be associated with susceptibility to viral infection and/or severity of disease in a population-based study but which are either in linkage disequilibrium with or in close physical proximity to a marker in the CD45 gene shown to be associated with susceptibility to viral infection.

As would be readily apparent to persons skilled in the art of human genetics, "linkage disequilibrium" occurs between a marker polymorphism (e.g. a DNA polymorphism which is "silent") and a functional polymorphism (i.e. genetic variation which affects phenotype or which contributes to a genetically determined trait) if the marker is situated in close proximity to the functional polymorphism. Due to the close physical proximity, many generations may be required for alleles of the marker polymorphism and the functional polymorphism to be separated by recombination. As a result they will be present together on the same haplotype at higher frequency than expected, even in very distantly related people. As used herein the term "close physical proximity" means that the two markers/alleles in question are close enough for linkage disequilibrium to be likely to arise.

In a preferred embodiment the method of the invention comprises screening for the presence or absence in the human subject of the C77G mutation in the gene encoding CD45, wherein subjects having at least one mutant allele are scored as being susceptible to viral infection.

As will be illustrated in the accompanying

Example, a mutation (C to G transversion) in the fourth or "A" exon of the CD45 gene has been shown to be associated with HIV-1 infection. In addition, the C77G mutation has been found in a patient with common variable immunodeficiency with persistent viral infection and prolonged excretion of polio virus (this patient was previously described by Misbah *et al.*, Postgrad Med J, 1991, Vol: 67, 301-303) and in a patient infected with EBV (data not shown). Furthermore, the inventors have shown the C77G mutation to be present in patients diagnosed with haemophagocytic lymphohistiocytosis (HLH) (data not shown). Sporadic cases of HLH are often provoked by viral infection in childhood (Dreyer, *et al.*, Am J Pediatr Hematol Oncol, Vol: 13, 476). In addition work with transgenic mice expressing single CD45 isoforms indicates that those expressing high molecular weight isoforms show defective immune responses (unpublished data). Together these observations suggest that the C77G mutation is predictive of a general susceptibility or predisposition to viral infection.

In the context of the invention, the process of screening for the presence or absence of a mutation or allelic variant in the genome of an individual may advantageously comprise screening for the presence or absence in the genome of the subject of both the common or wild type allele and the variant or mutant allele or may comprise screening for the presence or absence of either individual allele, it generally being possible to draw conclusions about the genotype of an individual at a polymorphic locus having two alternative allelic forms just by screening for one or other of the specific alleles.

The step of screening for the presence or absence

of a mutation or allelic variant in the genome of a subject, also referred to herein as "genotyping", can be carried out using any suitable methodology known in the art and it is to be understood that the invention is in no way limited by the precise technique used to perform such genotyping.

Known techniques for the scoring of single nucleotide polymorphisms include mass spectrometry, particularly matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), single nucleotide primer extension and DNA chips or microarrays (see review by Schafer, A. J. and Hawkins, J. R. in Nature Biotechnology, Vol 16, pp33-39 (1998)). The use of DNA chips or microarrays could enable simultaneous genotyping at many different polymorphic loci in a single individual or the simultaneous genotyping of a single polymorphic locus in multiple individuals. SNPs may also be scored by DNA sequencing.

In addition to the above, SNPs are commonly scored using PCR-based techniques, such as PCR-SSP using allele-specific primers (described by Bunce M, *et al.*, Tissue Antigens, 1995; **50**: 23-31). This method generally involves performing DNA amplification reactions using genomic DNA as the template and two different primer pairs, the first primer pair comprising an allele-specific primer which under appropriate conditions is capable of hybridising selectively to the wild type allele and a non allele-specific primer which binds to a complementary sequence elsewhere within the gene in question, the second primer pair comprising an allele-specific primer which under appropriate conditions is capable of hybridising selectively to the variant allele and the same non allele-specific primer. A still further

PCR-based technique for scoring SNPs is PCR ELISA.

If the SNP results in the abolition or creation of a restriction site, as is the case with the C77G mutation in the CD45 gene, genotyping can be carried out by performing PCR using non-allele specific primers spanning the polymorphic site and digesting the resultant PCR product using the appropriate restriction enzyme (also known as PCR-RFLP). Restriction fragment length polymorphisms, including those resulting from the presence of a single nucleotide polymorphism, may be scored by digesting genomic DNA with an appropriate enzyme then performing a Southern blot using a labelled probe corresponding to the polymorphic region (see Molecular Cloning: A Laboratory Manual, Sambrook, Fritsch and Maniatis, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

The known techniques for scoring polymorphisms are of general applicability and it will be readily apparent to persons skilled in the art that known techniques may be adapted for the scoring of single nucleotide polymorphisms in the CD45 gene. In the case of the C77G mutation, the preferred technique for scoring this mutation is PCR followed by digestion of the PCR product with the enzyme MspI, as described in the accompanying Example. However, the invention is not intended to be limited to the use of this technique.

Genotyping is preferably carried out *in vitro*, and is most preferably performed on isolated genomic DNA prepared from a suitable tissue sample obtained from the subject under test. Most commonly, genomic DNA is prepared from a sample of whole blood, according to standard procedures which are well known

in the art.

In a further aspect the invention provides a method of screening a human subject for susceptibility to viral infection and/or pre-disposition to developing severe disease following viral infection which comprises evaluating the pattern of CD45 mRNA expression in the subject, wherein the presence of an abnormal pattern of CD45 mRNA expression associated with the presence of a C77G mutant allele of the gene encoding CD45 is taken as an indication that the subject is more susceptible to viral infection and/or more pre-disposed to developing severe disease following viral infection, as compared to subjects who do not carry a C77G mutation.

The term "abnormal pattern of CD45 mRNA expression associated with the presence of a C77G mutant allele of the gene encoding CD45" refers to the variant CD45 splicing phenotype described by Thude *et al.*, Eur J Immunol, 1995, Vol: 25(7), 2101-6 and shown to be associated with heterozygosity for the C77G mutation. Individuals homozygous for the C77G mutation are expected to show an exaggeration of the mRNA expression pattern observed in heterozygotes.

Suitable RNA analysis techniques which may be used to determine the pattern of CD45 mRNA expression in accordance with the invention include, but are not limited to, RT-PCR, starting from a sample of total or mRNA prepared from a tissue which expressed CD45 (e.g. PBLs), Northern blotting and RNAse protection.

In a still further aspect the invention provides a method of screening a human subject for susceptibility to viral infection and/or pre-disposition to developing severe disease following

viral infection which comprises evaluating the pattern of CD45 protein expression in the subject, wherein the presence of an abnormal pattern of CD45 protein expression associated with the presence of a C77G mutant allele of the gene encoding CD45 is taken as an indication that the subject is more susceptible to viral infection and/or more pre-disposed to developing severe disease following viral infection, as compared to subjects who do not carry a C77G mutation.

The term "pattern of CD45 protein expression associated with the presence of a C77G mutant allele of the gene encoding CD45" refers to the variant pattern of expression of CD45 protein isoforms on peripheral T cells shown to be associated with heterozygosity for the C77G mutation, as described by Thude *et al.*, Eur J Immunol, 1995, Vol: 25(7), 2101-6. The normal pattern of CD45 protein expression is characterised by loss of expression of the CD45RA isoform and gain in expression of CD45RO after T cell activation. Individuals heterozygous for C77G are characterised continuous expression of the CD45RA isoform on activated and memory T cells, i.e. the T cells remain CD45RA/RO double positive after activation. Individuals homozygous for the C77G mutation are expected to show very little expression of CD45RO at the cell surface.

Analysis of the CD45 protein isoform expression pattern on peripheral T cells is preferably carried out using flow cytometry, as described in the accompanying example. Individuals heterozygous for C77G are characterised by the absence of a CD45RA negative population of leucocytes. Further suitable techniques which may be used to assess the pattern of expression of CD45 isoforms include immunoprecipitation and Western blotting.

The screening methods of the invention may be used to identify human subjects who are susceptible or pre-disposed to viral infection by virtue of their genetic make-up. This may allow intervention with preventative therapies aimed at boosting immune function. Screening for increased susceptibility to viral infections and/or for risk of developing more severe virus-induced disease would be important for individuals at increased risk of life threatening virus infections. These may include, for example, gay men and intravenous drug users or medical personnel working in renal dialysis units. "At risk" individuals may be counselled or excluded from high risk situations and measures may be taken to ensure that vaccination results in protective antibody titres in these individuals where a vaccine is available. Screening may also be useful for predicting whether individuals with chronic viral infection, such as for example Hepatitis B or C, are likely to be refractory to expensive immunotherapy.

The inventors have also made observations which provide evidence that carriage of the C77G mutation is predictive of susceptibility to immunodeficiency diseases other then severe combined immune deficiency (SCID) and/or a pre-disposition to developing more severe disease. Evidence for an association between C77G and predisposition to developing more severe immunodeficiency disease is provided in particular by the inventors' observation that the C77G mutation is present in a patient with common variable immunodeficiency and prolonged excretion of polio virus.

Therefore, in a still further aspect the invention provides a method of screening a human subject for susceptibility to developing

immunodeficiency disease other than severe combined immune deficiency and/or pre-disposition to a developing a more severe form of the immunodeficiency disease, which method comprises screening for the presence or absence in the genome of said subject of one or more polymorphic variants or mutations in the gene encoding CD45 or of one or more polymorphic variants in linkage disequilibrium with or in close physical proximity to a polymorphic locus in the gene encoding CD45.

In a preferred embodiment this method comprises screening for the presence or absence in the human subject of the C77G mutation in the gene encoding CD45, wherein subjects having at least one mutant allele are scored as being more susceptible to developing immunodeficiency and/or more likely to develop a severe form of immunodeficiency than subjects who do not carry a C77G mutation.

The invention further provides a method of screening a human subject for susceptibility to developing immunodeficiency disease other than severe combined immune deficiency and/or pre-disposition to a developing a more severe form of the immunodeficiency disease, which method comprises evaluating the pattern of CD45 mRNA expression in the subject, wherein the presence of an abnormal pattern of CD45 mRNA expression associated with the presence of a C77G mutant allele of the gene encoding CD45 is taken as an indication that the subject is more susceptible to developing immunodeficiency and/or more likely to develop a severe form of immunodeficiency than subjects who do not carry a C77G mutation.

The invention further provides a method of screening a human subject for susceptibility to

developing immunodeficiency disease other than severe combined immune deficiency and/or pre-disposition to a developing a more severe form of immunodeficiency disease, which method comprises evaluating the pattern of CD45 protein expression in the subject, wherein the presence of an abnormal pattern of CD45 protein expression associated with the presence of a C77G mutant allele of the gene encoding CD45 is taken as an indication that the subject more susceptible to developing immunodeficiency and/or more likely to develop a severe form of immunodeficiency than subjects who do not carry a C77G mutation.

In the context of this application the term "immunodeficiency disease other than severe combined immune deficiency" encompasses, but is not limited to, common variable immunodeficiency, selective IgA and/or IgG deficiency, DiGeorge syndrome, X-linked lymphoproliferative syndrome, Bloom's syndrome and Ataxia telangiectasia.

Tchilian *et al.* (J. Immunol., 2001, 166: 1308-1313) have previously reported a homozygous 6-bp deletion in the CD45 gene in a patient diagnosed with SCID. However, there have been no published reports of the significance of CD45 mutations in clinically milder immunodeficiencies such as, for example, common variable immunodeficiency, selective IgA and/or IgG deficiency, DiGeorge syndrome, X-linked lymphoproliferative syndrome, Bloom's syndrome, Ataxia telangiectasia. In particular, there has been no previous suggestion that C77G heterozygotes exhibit increased susceptibility to immunodeficiency and/or increased disease severity. By definition SCID is a very severe form of immunodeficiency, hence the presence of a C77G mutation in addition to the

causative SCID abnormality is unlikely to have a significant effect on disease severity.

The above methods of screening for susceptibility to developing immunodeficiency disease and/or pre-disposition to a developing a more severe form of immunodeficiency disease may be carried out using the same methodology as described previously for the screens for determining susceptibility to viral infection and/or pre-disposition to developing severe viral disease.

The invention will be further understood with reference to the following, non-limiting, Experimental Example and the accompanying Figures in which:

Figure 1 shows the results of FACS analysis to investigate the pattern of CD45 expression in human peripheral T cells pre- and post- stimulation. PBMC were stimulated with 1 µg/ml PHA and on days 0 and day 10 stained with isoform-specific CD45RO-PE and CD45RA-FITC antibody conjugates and with a CD3-APC antibody conjugate. Analysis was performed on gated CD3+ cells. Panels (A) and (B) show the normal pattern of CD45 expression pre- and post- stimulation: T cell activation is associated with a loss in CD45RA and a gain in expression of CD45RO. Panels (C) and (D) show the pattern of CD45 expression pre- and post-stimulation in a C77G heterozygote: the CD45RA population is largely absent and the T cells remain CD45RA/RO double positive after activation.

Example 1-Association between C77G and HIV infection

Genomic DNA samples and cryopreserved PBMC were obtained from 182 HIV-1 infected patients enrolled at

the St Stephen's Clinic, Chelsea and Westminster Hospital, as a part of a functional immunological study. An additional 15 DNA samples from individuals identified as HIV-1-infected at seroconversion, were supplied by Dr P. Borrow. Ethical approval was obtained and the patients gave written consent. The control group consisted of 236 healthy volunteer blood donors, obtained through the local blood bank of the UK National Blood Transfusion Service.

The detection of exon A (C77G) was performed on genomic DNA amplified by PCR using forward (5'-GACTACAGCAAAGATGCCCAGTG-3') and reverse primers (5'- GGGATACTTGGGTGGAAGTA-3'). The C77G transition introduces a new restriction site for Msp I, which cleaves the mutant PCR product into two fragments of 72 and 83 bp. The presence of an undigested band of 155 bp indicates the presence of the wild type allele.

The presence of the CD45 exon A mutant allele was confirmed by sequencing and flow cytometric analysis on C77G positive samples. PBMC were stimulated with PHA and on days 0 and day 10 stained with isoform specific CD45RO-PE and CD45RA-FITC antibody conjugates (obtained from Dako and Sigma, respectively) together with CD3-APC antibodies (obtained from Pharmingen). Analysis was performed on gated CD3+ T cells. The normal pattern of CD45 splicing is characterised by loss of CD45RA and gain in expression of CD45RO associated with the activated/memory function (A and B, Fig. 1). Variant CD45 splicing can be identified by the absence of the single CD45RO+ population and even after 10 days of stimulation the T cells remain CD45RA/RO double positive (C and D, Fig. 1).

Using PCR and Msp I digestion analysis 11 individuals with the exon A (C77G) mutation were identified out of

197 HIV-1 patients and 4 out of 236 healthy donors (Table 1). The presence of the C77G mutation in these individuals was confirmed by flow cytometric analysis of CD45 protein expression. Using two-tailed Fisher's exact test to test for the association between C77G mutation and HIV-1 infection, a statistically significant association was demonstrated ($p = 0.03$).

The results of this study clearly indicate that exon A (C77G) transversion and abnormal CD45 splicing are associated with HIV-1 infection.

Table I. Frequency of CD45 exon A (C77G) mutation in HIV patients and healthy controls

	Total number	Number with Exon A (C77)	Frequency
HIV	197	11	5.6%
Healthy donors	236	4	1.7%

GenBank Accession Numbers

M23461	human PTPRC gene exons 1 and 2
M23462	human PTPRC gene exon 3
M23494	human PTPRC gene exon 4
M23495	human PTPRC gene exon 5
M23496	human PTPRC gene exon 6
M23466	human PTPRC gene exon 7
M23467	human PTPRC gene exon 8
M23468	human PTPRC gene exon 9
M23469	human PTPRC gene exon 10

	M23470	human PTPRC gene exon 11
	M23471	human PTPRC gene exon 12
	M23472	human PTPRC gene exon 13
	M23473	human PTPRC gene exon 14
5	M23474	human PTPRC gene exon 15
	M23475	human PTPRC gene exon 16
	M23476	human PTPRC gene exon 17
	M23477	human PTPRC gene exon 18
	M23478	human PTPRC gene exon 19
10	M23479	human PTPRC gene exon 20
	M23480	human PTPRC gene exon 21
	M23481	human PTPRC gene exon 22
	M23482	human PTPRC gene exon 23
	M23483	human PTPRC gene exon 24
15	M23484	human PTPRC gene exon 25
	M23485	human PTPRC gene exon 26
	M23486	human PTPRC gene exon 27
	M23487	human PTPRC gene exon 28
	M23488	human PTPRC gene exon 29
20	M23489	human PTPRC gene exon 30
	M23490	human PTPRC gene exon 31
	M23491	human PTPRC gene exon 32
	M23492	human PTPRC gene exon 33

CLAIMS:

1. A method of screening a human subject for susceptibility to viral infection and/or pre-disposition to developing severe disease following viral infection, which method comprises screening for the presence or absence in the genome of the subject of one or more polymorphic variants or mutations in the gene encoding CD45 or of one or more polymorphic variants in linkage disequilibrium with or in close physical proximity to a polymorphic locus in the gene encoding CD45.

2. A method according to claim 1 which comprises screening for the presence or absence in the human subject of the C77G mutation in the gene encoding CD45, wherein subjects having at least one mutant allele are scored as being more susceptible to viral infection and/or more pre-disposed to developing severe disease following viral infection, as compared to subjects who do not carry a C77G mutation.

3. A method of screening a human subject for susceptibility to viral infection and/or pre-disposition to developing severe disease following viral infection which comprises evaluating the pattern of CD45 mRNA expression in the subject, wherein the presence of an abnormal pattern of CD45 mRNA expression associated with the presence of a C77G mutant allele of the gene encoding CD45 is taken as an indication that the subject is more susceptible to viral infection and/or more pre-disposed to developing severe disease following viral infection, as compared to subjects who do not carry a C77G mutation.

4. A method of screening a human subject for susceptibility to viral infection and/or pre-

disposition to developing severe disease following viral infection which comprises evaluating the pattern of CD45 protein expression in the subject, wherein the presence of an abnormal pattern of CD45 protein expression associated with the presence of a C77G mutant allele of the gene encoding CD45 is taken as an indication that the subject is more susceptible to viral infection and/or more pre-disposed to developing severe disease following viral infection, as compared to subjects who do not carry a C77G mutation.

5. A method according to any one of claims 1 to 4 wherein the viral infection is infection with a human immunodeficiency virus.

6. A method according to claim 5 wherein the human immunodeficiency virus is HIV-1.

7. A method according to any one of claims 1 to 4 wherein the viral infection is infection with EBV.

8. A method according to any one of claims 1 to 4 wherein the viral infection is infection with poliovirus.

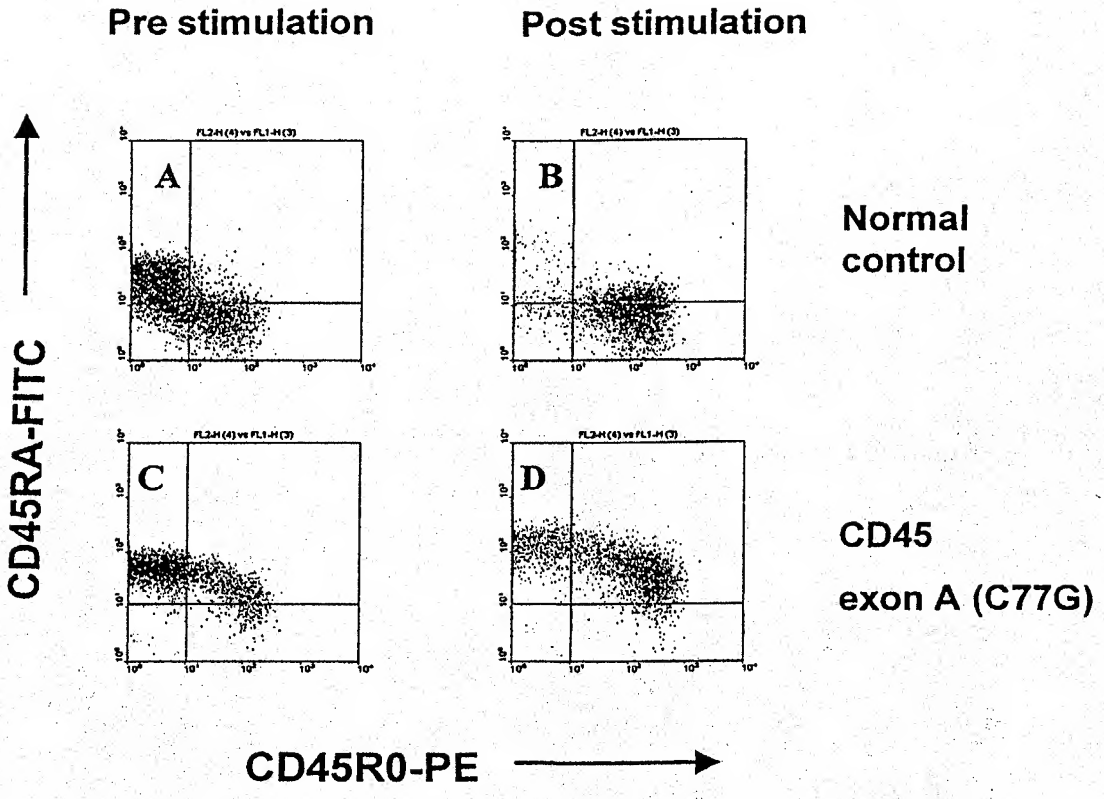
9. A method of screening a human subject for susceptibility to developing immunodeficiency disease other than severe combined immune deficiency and/or pre-disposition to a developing a more severe form of immunodeficiency disease, which method comprises screening for the presence or absence in the genome of said subject of one or more polymorphic variants or mutations in the gene encoding CD45 or of one or more polymorphic variants in linkage disequilibrium with or in close physical proximity to a polymorphic locus in the gene encoding CD45.

10. A method as claimed in claim 9 which comprises screening for the presence or absence in the human subject of the C77G mutation in the gene encoding CD45, wherein subjects having at least one mutant allele are scored as being more susceptible to developing immunodeficiency and/or more likely to develop a severe form of immunodeficiency than subjects who do not carry a C77G mutation.

11. A method of screening a human subject for susceptibility to developing immunodeficiency disease other than severe combined immune deficiency and/or pre-disposition to a developing a more severe form of immunodeficiency disease, which method comprises evaluating the pattern of CD45 mRNA expression in the subject, wherein the presence of an abnormal pattern of CD45 mRNA expression associated with the presence of a C77G mutant allele of the gene encoding CD45 is taken as an indication that the subject is more susceptible to developing immunodeficiency and/or more likely to develop a severe form of immunodeficiency than subjects who do not carry a C77G mutation.

12. A method of screening a human subject for susceptibility to developing immunodeficiency disease other than severe combined immune deficiency and/or pre-disposition to a developing a more severe form of immunodeficiency disease, which method comprises evaluating the pattern of CD45 protein expression in the subject, wherein the presence of an abnormal pattern of CD45 protein expression associated with the presence of a C77G mutant allele of the gene encoding CD45 is taken as an indication that the subject more susceptible to developing immunodeficiency and/or more likely to develop a severe form of immunodeficiency than subjects who do not carry a C77G mutation.

Fig. 1


Pre stimulation
Post stimulation
A
B
C
D
Normal control
CD45
exon A (C77G)
CD45RA-FITC
CD45R0-PE